AMENDMENTS TO THE SPECIFICATION

Please insert the following paragraph before the technical field section of the specification:

This application claims priority benefit, under 35 U.S.C. § 119(a), of Japanese Patent Application No. 2001-112367, filed April 11, 2001.

Please amend paragraph 0024 as follows:

This will be explained in more detail by taking as an example the case of a search for a drug useful in effecting recovery from damage due to ischemia. In an ischemia model animal, a gene having an expression level that differs between before and after an event, being ischemia, is detected with a DNA microarray or DNA chip (for example, ~~GeneChip™~~ the high-density oligonucleotide array GENECHIP™ (U.S. Affymetrics, Inc.)) (genome-wide screening). Next, sequence information of the genes having expression levels that differ between before and after the ischemia event is obtained, by linking data obtained by DNA microarray or DNA chip with bioinformatics. Based on this sequence information, a probe for in situ hybridization is designed, and prepared. Thereafter, in situ hybridization is used to examine how this gene is distributed in what types of tissue in an organ in which ischemia occurred (for example, brain, liver, etc.). In situ hybridization operations, can be either manual or automatic. For example, by using Ventana HX system (Ventana Medical Systems, Inc.) which realizes a complete automation of in situ hybridization, results with good reproducibility can be obtained in a short time. From the results of in situ hybridization, it is possible to screen a gene which has a tissue distribution thought to be suitable for its use a drug. For example, where a drug for memory recovery is being sought, a gene having expression localized in the hippocampus is selected. Further, where a drug which suppresses inflammation is being sought, a gene the expression of which is distributed in the entire brain is selected.

Please amend paragraph 0027 as follows:

A method of analysis using conventional blotting techniques, wherein hybridization is performed simultaneously in respect of a plurality of probes arranged in an array, is generally referred to as array technology (The chipping forecast. Nature Genetics, supplement vol. 21, (1999)). In particular, where an array is prepared with probes as spots in an array form having a diameter of less that 1 mm, this is referred to as a microarray or chip, and arrays having probes constituted by DNA are referred to as "DNA chips". At present, methods realizing this

array technology include a method where cDNA is spotted on a filter to form an array, a method here cDNA or synthetic DNA is spotted on a slide glass, and further methods such as ~~GeneChip™~~ the high-density oligonucleotide array GENECHIP™ (U.S. Affymetrics, Inc.) (Lockhart, D. J. et al. (1996), Expression monitoring by hybridization to high-density oligonucleotide arrays. Nature Biotechnology 14, 1675-80; Wodicka, L. et al. (1997) Genome-wide expression monitoring in Saccharomyces cerevisiae. Nature Biotechnology 15, 1359-67). In ~~GeneChip™~~ GENECHIP™, spotting technology differs from that of other DNA chips. To distinguish between this and other methods, this method per se is referred to by the trademark ~~"GeneChip"~~ "GENECHIP™". With ~~GeneChip™~~ GENECHIP™, it is possible to perform both genome analysis for analyzing genome DNA mutation, and expression analysis for analyzing gene expression. However, in the present invention, expression analysis is performed.

Please amend paragraph 0029 as follows:

In the ~~GeneChip~~ GENECHIP™ technique, the method of spotting DNA probes on the chip differs from that of other DNA chips. In conventional methods, a DNA probe directly excised from an organism was spotted on a foundation. In contrast, with the ~~GeneChip~~ GENECHIP™ technique, DNA is synthesized as fragments of 18 to 25 mer using a photochemical reaction in a step resembling semiconductor manufacture techniques. As a result, several million probes having 18 to 25 mer nucleotide sequences are immobilized on a 50 or 24 μm square probe cell (this is referred to as a "tile").

Please amend paragraph 0033 as follows:

(2) ~~GeneChip™~~ GENECHIP™ is constituted by a hybridization oven for binding the sample to the probes on the chip, a Fluidic Station for washing and labeling, a Gene Array scanner for, reading fluorescence emissions, and a computer system for processing and analyzing the read information. Further, since experimental conditions from sample preparation to data collection are optimized by using a pre-existing kit, it is possible to obtain data with high reproducibility. Since the expression levels of several thousand genes are precisely assayed on a chip, arranging probes for E. coli genes on the chip and mixing a fixed amount of cRNA derived from E. coli genes in with the sample as a control (spiking) enables quantitivity of the genes within the sample to be raised, as well as providing a check on the precision of the operating process of the experiment. Further, by using in conjunction with the results of measurement using probes for housekeeping genes such as GAPDH and actin,

comparative analysis of a plurality of different experimental results can be conducted, and reliable data having a wide dynamic range, can be obtained with high sensitivity without the user being troubled by examination of experimental conditions, etc.

Please amend paragraph 0035 as follows:

~~GeneChip™~~ GENECHIP™ is a comprehensive system encompassing steps from sample preparation to data analysis, and is almost fully completed. Consequently, for reagents, kits, etc. to be used in each step, the following, which are recommended by Affymetrics, Inc., are recommended here.

Please amend paragraph 0065 as follows:

Since a large amount of data can be obtained with ~~GeneChip™~~ GENECHIP™, to use it efficiently, so-called bioinformatics (Bioinformatics) techniques are required. For this purpose, in ~~GeneChip™~~ GENECHIP™, as bioinformatics tools, the proprietary ~~GeneChip~~ GENECHIP™ Laboratory Information Management System (LIMS™) and ~~GeneChip~~ GENECHIP™ Expression Data Mining Tool (EDMT™) are provided therewith, and these enable data to be input into a SQL compliant database in a format determined by an open consortium for standardizing gene-related analysis techniques (GATC), and linked to gene information databases (GenBank, etc.) published on the internet. However, since bioinformatics per se is still in a developmental stage, there are cases where data analysis with known systems is insufficient. In small and medium scale research facilities, there arises the need to separately file and analyze the databases of a few individuals, and use other analysis programs to perform data processing, graphing, and statistical calculations. Here, the present inventors naturally access an LIMS-SQL server and use an EDMT-like tool to process data, store individual data in a GATC compatible extension database, and using Gene Spring (U.S. Silicon Genetics, Inc.) perform clustering, tabulation, searches, and information database searches. Further for statistical calculations, and analysis of the functional hierarchy of individual genes, the present inventors use Stingray (U.S. Affymetrics, Inc.).

Please amend paragraph 0143 as follows:

An adult mouse (male, Bcl black, purchased from Sankyo Lab Services) whose common carotid arteries were bilaterally ligated for 20 minutes to interrupt blood flow was adopted as a brain ischemia model. Thereafter, mice were euthanized after passage of time from recovery of blood flow (0 to 24 hours), the hippocampus removed, and a sample

prepared according to the protocol. Gene expression analysis was performed using a Mu6,500 Oligonucleotide DNA Probe array with the ~~GeneChip™~~ high-density oligonucleotide array GENECHIP™ system of Affymetrics, Inc. (U.S.). With Mu6,500 Oligonucleotide DNA Probe array, 6500 types of genome could be analyzed simultaneously. Table 1 shows results of analysis using bioinformatics (specifically, a scatter plot by LIMS-EDMT was used) on the basis of this data. The horizontal axis of FIG. 1 shows the genome expression level of a rat (control) in which ischemia processing was not conducted. The vertical axis indicates the genome expression level 24 hours after ischemia/reperfusion. The individual points in FIG. 1 correspond to respective specified genomes. It was possible to differentiate between those where expression level had increased (e.g., in *1, there was an increase in expression of approx. 20 times from 30 to 600 as between before and after ischemia), those for which there was no change (for example, *2), and those which were reduced (for example, in *3, there was a decrease in expression of a factor of approx. {fraction (1/100)} from 7,000 to 70 as between before and after ischemia, and thus we were able to know in general terms about the expression level in the tissue of specific genes. This result confirmed changes in expression level of approximately 1,000 types of genes. If this is connected with publicly available gene information databases, individual genome information can be instantaneously obtained.

Please amend paragraph 0144 as follows:

An adult rat (male 12-weeks old, Wister-type male rat purchased from Sankyo Lab Services) in which the hepatoportal portion was ligated for 15 minutes to interrupt blood flow, was adopted as a hepatic ischemia model. Thereafter, rats were euthanized after passage of time (0 to 4 hours) after recovery of blood flow, the liver removed and samples prepared according to the protocol. Gene expression analysis was performed using a Rat Toxicology U34 array with the ~~GeneChip™~~ high-density oligonucleotide array GENECHIP™ system of Affymetrics, Inc. (U.S.). With the Rat Toxicology U34 array, approximately 850 types of rat gene and EST could be analyzed simultaneously. FIG. 2 shows results of analysis using bioinformatics (specifically, a scatter plot by LIMS-EDMT was used) on the basis of this data. The vertical axis of FIG. 2 shows the genome expression level of a rat (control) in which ischemia processing was not conducted. The horizontal axis indicates the genome expression level 4 hours after ischemia/reperfusion. The individual points in FIG. 2 correspond to respective specified genomes. Examples of these include Hsc70 and TATase (Tyrosine aminotransferase) As a result, comparing between 0 hours and 4 hours, there were approximately 100 types where gene expression had increase 2 times or more, and

approximately 40 types where gene expression had fallen to 1/2 or less. In respect of Hsc70 and TATase, there was no dominant change in expression level according to ~~GeneChip™~~ GENECHIP™ was exhibited in respect of both genes as between the control group and the ischemia treated.

Please amend paragraph 0150 as follows:

Regarding Hsc70 and TATase, the expression level according to ~~GeneChip™~~ GENECHIP™ of both genes in both the control group, ischemia processed group exhibit no particular dominance. However, with in situ hybridization, it is clear that expression around the central ~~vain~~ vein increased as between before and after ischemia. Further, it was clear that this change was markedly appearing due to TATase. Thus, by combining ~~GeneChip™~~ GENECHIP™ and in situ hybridization, information regarding a greater number of genes can be obtained.

Please amend paragraph 0151 as follows:

An adult rat (male, Wister-Kyoto, 12-weeks old, purchased from Sankyo Lab Service) whose bilateral body temperature and brain temperature was maintained at 37.degree. C., whose common carotid arteries were bilaterally ligated for 10 minutes to interrupt blood flow, and further whose blood pressure was reduced to 30 to 40 mmHg, was adopted as a brain ischemia model (generally known as, Smith's brain ischemia model). After 10 minutes, reperfusion was allowed, and brain temperature and body temperature were maintained at 37°C. After 2 hours, the rat was euthanized, the hippocampus removed and a sample prepared following the protocol. Using rat U34 array, gene expression analysis was perform with (~~GeneChip™~~ GENECHIP™ system of Affymetrics, Inc. (United States). With a rat U34 array, it was possible to analyze 34,000 types of genome simultaneously. FIG. 7 shows results of analysis using bioinformatics (specifically, a scatter plot by LIMS-EDMT was used) on the basis of this data. FIG. 7, is a figure showing a scatter plot in respect of change in 34000 genes in a control group and 2 hours after ischemia. The horizontal axis of FIG. 7 shows the genome expression level of a rat (control) in which ischemia processing was not conducted. The vertical axis indicates the genome expression level 2 hours after ischemia/reperfusion. The individual points in Figure correspond to respective specified genomes. Examples of these include HSC70, HSP70, c-jun, EST1 and EST2. Points lying on the X=Y line indicate that there was no change in the corresponding genes between before and after processing. As a result, comparing between 0 hours and 2 hours, there were

approximately 475 types where gene expression had increase 2 times or more, and approximately 486 types where gene expression had fallen to 1/2 or less. In respect of HSC70, there was no change between before and after ischemia. (Intensity change Control*OH: approximately 30,000→ischemia*2H: approximately 30,000). HSP70 exhibited an increase in expression of as much as 20 times as between before and after ischemia (Intensity change Control*OH: approx. 1,000→ischemia*2H: approx. 20,000). c-jun was hardly expressed at all prior to ischemia but after ischemia there was a dramatic increase in the expression thereof (Intensity change Control*OH: approx. 0.1→ischemia*2H: approx. 20,000). EST1 was hardly expressed at all prior to ischemia but after ischemia there was a dramatic increase in the expression thereof (Intensity change Control*OH: approx. 3,000→ischemia*2H: approx. 15,000). EST2 exhibited an increase in expression of as much as 5 times as between before and after ischemia (Change in intensity Control*OH: approx. 3,000→ischemia *2H: approx. 15,000).

Please amend paragraph 0153 as follows:

Results for the control rat and brain ischemia model rat are shown in FIG. 8. Within the figures, anti-sense shows the results of staining with antisense probe, and sense indicates results of staining with sense probe. Diffuse expression Of HSP70 in the whole brain had increased. In particular, expression thereof increased markedly in the hippocampus. This matched with ~~GeneChip™~~ GENECHIP™ data and therefore provides support for the data obtained with ~~GeneChip™~~ GENECHIP™.

Please amend paragraph 0155 as follows:

Results for the control rat and the brain ischemia model rat are shown in FIG. 9. Within the figures, anti-sense shows the results of staining with antisense probe, and sense indicates results of staining with sense probe. Diffuse expression of c-jun in the whole brain was increasing. This, in respect of the point that there had been no expression, but as a result of ischemia, there was a dramatic increase, matched with ~~GeneChip™~~ GENECHIP™ data and therefore provides support for the data obtained with ~~GeneChip™~~ GENECHIP™.